# CHAPTER 8

# COLIFORM BACTERIA

## 8.1 INTRODUCTION

Coliform concentrations in natural waters have been used as an indicator of potential pathogen contamination since at least the 1890's (Whipple, 1917). Until recently, coliforms have been considered to be less sensitive to environmental stresses than enteric pathogens. Accordingly, coliforms were believed to be more persistent in natural waters and, therefore, a "safe" or conservative index of potential pathogen levels.

However, recent evidence about enteric viruses, opportunistic pathogens, and pathogenic Escherichia coli have raised doubts that coliforms are the "ideal indicator" (Sobsey and Olson, 1983). First, enteric viruses appear to generally have both lower decay rates than coliforms and also a lower ID-50 (i.e., the dose required to infect 50 percent of the persons exposed) than most bacterial enteric pathogens. Second, opportunistic pathogens (e.g., Pseudomonas aeruginosa, Aeromonas hydrophila, and Legionella pneumophila) often have major non-fecal sources and are able to grow in natural waters. These pathogens generally have a high ID-50, threatening primarily immunologically compromised persons such as hospital patients who are being given immunological suppressants. Finally, some strains of E. coli produce an enteric toxin that results in gastroenteritis.

In the context of drinking water, Olivieri (1983) has recommended that different indicators be used when different aspects of pathogen behavior are of interest, e.g., indicator of feces, treatment efficiency, or post-treatment contamination. Chamberlin (1982) has compared coliform (combining Total Coliform, Fecal Coliform, and E. coli) decay rates with pathogen and

virus decay rates measured simultaneously and has found that the respective decay rates were highly correlated ($r^2$ = 0.73) and that within-species variability was as great as pathogen-to-coliform variation (see Figure 8-1). At low decay rates, coliform decay rates were approximately equal to pathogen decay rates while at the highest decay rates, pathogen decay was slower.

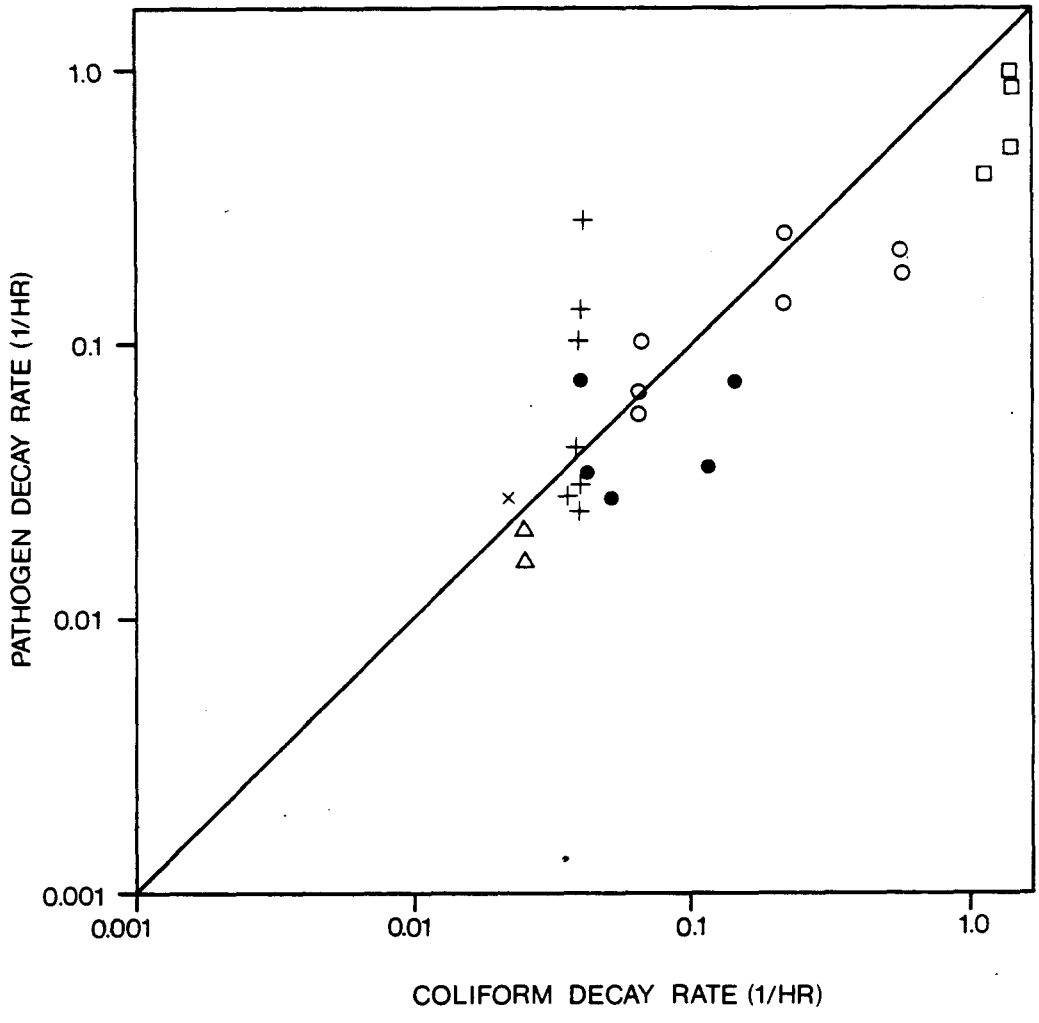


Figure 8-1. Relationship between pathogen or virus decay rates and coliform decay rates based on figure presented by Chamberlin (1982). Decay rates were estimated by Chamberlin based on data from Baross et al. (1975) (△), Morita (1980) (X), McFeters et al. (1974) (+), McCambridge and McMeekin (1981) (○), Lantrip (1983) (●), and Kapuscinski and Mitchell (1981) (□). The line shown represents coliform decay rates equal to pathogen decay rates.

In addition, epidemiological studies have revealed that enterococci levels are more closely associated with enteric disease than are coliforms (Cabelli et al. 1982). This work has in part motivated a proposed revision of the contact recreation bacterial water quality criteria: switching from fecal coliforms to E. coli and/or enterococci (U.S. Environmental Protection Agency, 1984).

Taken as a whole, these issues may serve to motivate modelers to include additional indicators as state variables and to use coliforms as an indicator rather than as the indicator.

## 8.2 COMPOSITION AND ASSAY

The coliform group consists of both fecal and non-fecal components. The fecal component includes mainly the Escherichia and Klebsiella genera while the non-fecal component includes mainly the Enterobacter and Citrobacter genera commonly associated with soils and plants (Dufour, 1977).

Neither the multiple tube (MPN) nor the membrane filter (MF) techniques for Total Coliforms (TC) effectively differentiates between the fecal and non-fecal components. The Fecal Coliform (FC) tests (either MPN or MF) provide a better differentiation at the cost of additional labor and time plus more exacting equipment requirements. The tests require either supplemental tests run on TC or incubation at elevated temperatures within precise limits (i.e., $44.5^{o}C \pm 0.2^{o}C$). These more stringent conditions eliminate most of the non-fecal component while still permitting the fecal component to survive. FC represents from 15 to 90 percent of the TC, depending on sample source. Unfortunately, there are major non-fecal sources of FC, most commonly of Klebsiella species (Hendry et al. 1982). Pulp mill wastewater provides a frequent example. Tests for E. coli are even more specific to fecal sources, but again incur further costs for labor and time.

The non-fecal components of the coliforms, especially the Enterobacter and Citrobacter genera, are of limited use in indicating fecal contamination

but do indicate prior contact with soil or plant material. In addition, these genera are capable of regrowth in nutrient-rich natural waters or where surfaces are available for growth.

Fecal streptococci (FS) provide another common indicator of fecal contamination (Clausen et al. 1977). Although all FS belong to the single genus Streptococcus, there are again fecal and non-fecal components. Enterococci and S. faecalis are more specific to fecal sources than the non-enterococcal streptococci. FS and particularly the enterococci are often considered to be able to survive longer in natural waters than either TC or FC. Chamberlin (1982) compared FS (combining TC, FC, and E. coli results) decay rates in cases where the rates were measured in the same experiments and found a high correlation ($r^2$ = 0.80) between the logarithm of the respective rates. In addition, the relationship between the logarithms of the rates had a slope estimated by linear regression that was not significantly different (p = 0.05) from 1.0. The intercept was marginally distinguishable from 0.0 at p = 0.01 and was estimated as -0.31. This suggests that coliform decay rates were generally twice as large as FS decay rates but that the rates changed generally by equal amounts from one environment to another. According to Geldreich and Kenner (1969), the FC/FS ratio is useful in discriminating between recent human versus animal fecal contamination. If the ratio exceeds approximately 1 (although 4 is often cited as the cut-off value), the source is presumptively human fecal material while if the ratio is less than 1, the source is assumed to be animal feces. But as Dutka and Kwan (1980) have observed, the ratio can change dramatically once the material enters natural waters. They monitored changes from an initial ratio of 2.7 to a low of 0.07 and a high of 22.5 in a single experimental run.

Other proposed fecal indicators have been discussed by Olivieri (1983) and include Clostridium perfringens, yeasts, and RNA coliphages. None of these novel indicators has become generally accepted.

Beyond the selections of a particular indicator or set of indicators, recent work has shown the importance of sublethal stress or injury of influencing observed concentrations in decay studies (Rose et al. 1975;

Bissonette et al. 1977). Rhodes and Kator (1982) and Kapuscinski and Mitchell (1981) have, among others, substantiated these results and have suggested particular mechanisms of injury. Consequently, the decision to use or not use a resuscitation step (e.g., incubation at 35°C in less selective medium for two hours) can have a major impact on the observed decay rates.

## 8.3 MODELING COLIFORMS

Modeling of coliforms is done for one main reason--establishing the level of fecal and/or soil pollution and potential pathogen contamination. The usual approach is simply to simulate disappearance and to estimate coliform levels as a function of initial loading and the disappearance rate which, in turn, is a function of time or distance of travel from the source and of environmental conditions such as temperatures, salinity, and light intensity.

### 8.3.1 Factors Affecting Disappearance Rates

Upon discharge to a water body, environmental conditions determine the extent to which coliform regrowth and death occur. Fecal coliforms and streptococci are occasionally observed to increase in numbers, although this may be due to disaggregation of clumps of organisms. Non-fecal organisms may, in fact, increase in numbers in natural waters where conditions are adequate (Lombardo, 1972; Mitchell and Chamberlin, 1978).

Factors can be conveniently classified into three categories: physical, physicochemical, and biochemical-biological. However, note that synergisms (e.g., osmotic effects and photo-oxidation) and interferences (e.g., sedimentation versus photo-oxidation) may exist. Kapuscinski and Mitchell (1980) and Bitton (1980) have reviewed factors that govern virus inactivation in natural waters and present essentially a parallel list to the one given below.

Physical factors that can affect the coliform population in natural waters, resulting in an apparent increase or decrease in the coliform disappearance rate include:

- Photo-oxidation
- Adsorption
- Flocculation
- Coagulation
- Sedimentation
- Temperature

Physicochemical factors include

- Osmotic effects
- pH
- Chemical toxicity
- Redox potential

Biochemical-biological factors include:

- Nutrient levels
- Presence of organic substances
- Predators
- Bacteriophages (viruses)
- Algae
- Presence of fecal matter

8.3.1.1 Physical Factors

Chamberlin and Mitchell (1978) have noted that, although many data have been collected on coliform disappearance rates, mechanisms mediating the rates have historically been poorly understood. According to Chamberlin and Mitchell, however, light is one of the most important factors. They observe that it is difficult to show statistically significant relationships between coliform disappearance rates and many factors usually hypothesized as

influencing those rates. In contrast, significant relationships between light intensity and coliform disappearance rates can be demonstrated. Chamberlin and Mitchell (1978) have shown that field data statistically support the photo-oxidation model (to be discussed), and data presented by Wallis et al. (1977) also appear to implicate incident light. Subsequent work by Sieracki (1980), Kapuscinski and Mitchell (1983), Lantrip (1983), and others has demonstrated that viruses and enteric bacterial pathogens are also sensitive to light but that viruses are generally less sensitive than coliforms.

Chamberlin and Mitchell (1978) have elaborated upon possible mechanisms by which light may increase coliform disappearance rates. They point out that although in many cases of light induced mortality, one or more photosensitizing substances are involved, visible and near ultraviolet (UV) light can kill E. coli in the absence of exogenous photosensitizers. Grigsby and Calkins (1980) have confirmed the significance of the near UV.

One suggested mechanism is that light quanta drive some exogenous or endogenous chromophore to an electronically excited state. The chromophore, in the process of returning to the ground state, transfers its absorbed light energy to another substance to form superoxides ($O_2^*$), which, in turn, cause damage to cellular components. Alternatively, the activated chromophore may cause damage directly, without the agency of a superoxygenated intermediate. Kapuscinski and Mitchell (1981) observed that injury to the catalase system is the most likely site of damage in E. coli and that the damage can be repaired if the coliforms are transferred to an appropriate recovery medium. Krinsky (1977) has, on the other hand, argued that the "cause of death" may be division-inhibition, mutation, and/or membrane damage.

Substances within coliform and other bacterial cells are effective, near-UV chromophores, including ubiquinones, porphyrins, and tryptophan (Krinsky 1977). Exogenous sources of photo-oxidants include algal pigments, lignins, and humic and fulvic acids. More highly colored and turbid waters have been shown to produce peroxides, singlet oxygen, and hydroxide radicals

at greater rates than well waters (for example, Zepp et al. 1977; Cooper and Zika, 1983).

Adsorption, coagulation, and flocculation may affect coliform disappearance rates, although few quantitative data are available. Adsorption refers to the attachment of coliform organisms to suspended particles. Coagulation refers to the coalescence of bacteria into clumps, and flocculation refers to the formation of soft, loose aggregates incorporating much water.

According to Mitchell and Chamberlin (1978), early investigations by several workers have demonstrated that clays tend to adsorb coliforms more than do silts or sands. This is, of course, commonly the case with sorbed substances. As Mitchell and Chamberlin point out, the nature and stability of coliform aggregates incorporating other particulate matter depends to a very large extent upon the physicochemical nature of the particles. Gannon et al. (1983) found that 90 to 96 percent of the coliforms entering a lake from upland watersheds were associated with 0.45 to 5 $\mu$m particles.

Sedimentation involves the settling out of bacterial particles and aggregates. The rate of disappearance may be materially influenced by aggregation and sedimentation, but the magnitude and direction of the change in rate is not well understood. The mechanism of apparent disappearance due to sedimentation is actually simple removal of cells from the water column--that is, transfer of matter from one physical compartment (the water column) to another (the benthos). However simple, sedimentation may sometimes be the predominant mechanism of removal as Gannon et al. (1983) demonstrated in a field study of coliform survival in a lake. Accordingly, modeling coliform disappearance in the water column may give misleading results, particularly where shellfish are harvested for human consumption. Reduction in coliform levels in the water column may simply mean increased numbers in the benthos.

Temperature influences most, if not all, of the the other factors. Bitton (1980) and Lantrip (1983) argue that temperature is the single most important modifier of decay rates, especially in freshwater and in the dark.

### 8.3.1.2 Physicochemical Factors

Mitchell and Chamberlin (1978) report that physicochemical factors may have significant effects on disappearance rates. Survival rates of E. coli, for example, are inversely proportional to salinity both in natural seawater (due to osmotic and other effects) and in artificial salt solutions. In addition, Sieracki (1980) has observed a synergism with light effects. Work by Zafiriou and True (1979) suggest that nitrite photolysis in seawater may be a partial cause. In general, E. coli have been found to survive longer in lower pH salt solutions (pH < 8) than under alkaline conditions.

Heavy metal toxicity toward microorganisms has been known since the late nineteenth century. A great number of studies have been done on the "oliogodynamic action" of silver and copper salts. According to Mitchell and Chamberlin (1978), heavy metals have been implicated as important mediators of E. coli disappearance rates, and the heavy metal effects may be reduced by addition of chelating agents. Redox potential, through its effect on heavy metals solubilities, also affects disappearance rates. In addition to this, redox may influence disappearance rates in other ways, although data on this are not extensive.

Finally, Kott (1982) has presented evidence that when coliforms undergo the transition from the generally low oxygen environment of sewage to the higher oxygen levels found in seawater, the oxygen shock promotes rapid decay.

### 8.3.1.3 Biochemical and Biological Factors

Nutrient concentrations may be important in determining disappearance rates under some conditions. In many nutrient studies, the apparent impact of nutrient addition to the coliform culture is due to chelation of heavy metal ions (Mitchell and Chamberlin, 1978). Thus, the apparent decrease in disappearance rate in many cases may not be due to the additional nutrient, but instead to reduce toxicity of the culture medium. Mitchell and Chamberlin (1978) cite the work of Jones (1964) who found that E. coli would

not grow at 37°C in either filter-sterilized natural or synthetic seawater supplemented with glucose, ammonium chloride, and potassium phosphate. Inhibition could be reversed by autoclaving, by addition of very small amount of organic matter, or by addition of metal chelating or complexing agents. Jones demonstrated that two levels of toxic metals would produce the inhibitory effect, and concluded that the apparent influence on disappearance rates was due to naturally occurring trace heavy metals in solution. Furthermore, as Mitchell and Chamberlin (1978) note, other researchers have obtained experimental results implicating heavy metals, and their chelation upon addition of nutrients, in apparent changes in disappearance rates.

In some situations, it appears that nutrient levels influence disappearance rates in ways unrelated to toxic metals availability. Savage and Hanes (1971) and Chamberlin (1977), for example, have reported growth-limiting effects of available BOD or organic matter. Recent work by Dutka and Kwan (1983) indicates that after-growth and long-term persistence is particularly sensitive to nutrient levels. Further, it is possible that the level of nutrients affects coliform predators, thereby influencing rates of grazing on coliforms. Mitchell and Chamberlin (1978) report that predators in natural waters may be significant in reducing coliform populations given high predator levels. They cite three groups of micro-organisms which may be importantly in seawater. These are cell wall-lytic marine bacteria, certain marine amoebae, and marine bacterial parasites similar to Bdellovibrio bacteriovorus. Experiments performed by a number of researchers have implicated predators in disappearance of coliforms in both fresh and seawater, although Lantrip (1983) did not observe a significant predator influence in chamber experiments using freshwater. Bacteriophages, on the other hand, are apparently of minor importance, despite their demonstrated presence in sea water. The relative insignificance of phages, according to Mitchell and Chamberlin (1978), stems from their ineffectiveness in killing E. coli where the bacterial cells are not actively growing and multiplying, and the rapid inactivation of the phages by seawater.

Some forms of phytoplankton produce antibacterial agents which are excreted into the water column. These substances are heat-liable macromolecules, and according to Mitchell and Chamberlin (1978) at least one, a chlorophyllide, is active only if the system is illuminated. The fact that at least one antibacterial agent is activated by light suggests that algae may play a mediating role in the effect of light on disappearance rates. Other mechanisms of algal anti-coliform activity have been suggested. One is that during algal blooms, other organisms which prey on both algae and coliforms may also increase in numbers.

Table 8-1 is a summary of factors influencing coliform disappearance rates.

### 8.3.2 Modeling Formulations

Traditionally, coliform modeling has only taken into account disappearance, and a simple first-order kinetics approach has been used (Baca and Arnett,1976; Chen, et al., 1975; Chen et al., 1976; U.S. Army Corps of Engineers,1974; Chen and Orlob, 1975; Lombardo, 1973; Lombardo, 1972; Smith, 1978; Anderson et al. 1976; Huber, et al. 1972; Hydroscience, 1971; Chen and Wells, 1975; Tetra Tech, 1976b):

$$\frac{dC}{dt} = -kC \tag{8-1}$$

or

$$C_t = C_o e^{-kt} \tag{8-2}$$

where $C$ = coliform concentration, MPN or count/100 ml
$C_o$ = initial coliform concentration, MPN or count/100 ml
$C_t$ = coliform concentration at time t, MPN or count/100 ml
$k$ = disappearance rate constant, $day^{-1}$ or $hr^{-1}$
$t$ = exposure time, days or hours.

A summarized listing of values for k is presented in Table 8-2. The data summarize 30 studies of rates measured *in situ*. Table 8-3 shows values for k from a number of modeling studies. The median rate for the *in situ* studies is .04 $hr^{-1}$ with 60 percent of the values less than .05 $hr^{-1}$ and 90 percent less than .22 $hr^{-1}$.

TABLE 8-1. FACTORS AFFECTING COLIFORM DISAPPEARANCE RATES

| Factor | Effects |
|---|---|
| Sedimentation | Important with regard to water column coliform levels, particularly where untreated or primary sewage effluent or stormwater is involved, and under low vertical mixing conditions. May adversely affect shellfish beds by depositing coliforms and fecal matter into benthos. |
| Temperature | Probably the most generally influential factor modifying all other factors. |
| Adsorption, Coagulation, Flocculation | Inconclusive. |
| Solar Radiation | Important; high levels may cause more than 10-fold increase in disappearance rate over corresponding rate in the dark in seawater. Rates also materially increased in freshwater. |
| Nutrient Deficiencies | Appear to accelerate disappearance. Numerous studies have indicated that increasing nutrient levels of seawater decrease disappearance rates. |
| Predation | Several species of organisms (bacteria, amoebae) have been shown to attack and destroy *E. coli*. Importance of predation depends strongly on the concentration of predators. |
| Bacteriophages | Apparently not important. |
| Algae | Bactericidal substances are known to be produced by planktonic algae. Substances may be photoactivators, mediating the influence of light on coliform disappearance. This might account for variability of data in studies of light-induced disappearance rates. Another hypothesis is that algal predators with blooms concomitant with algal blooms may produce substances toxic to *E. coli* or may prey upon them. |
| Bacterial Toxins | Antibiotic substances produced by indigenous bacteria are not believed important in coliform disappearance. |
| Physiochemical Factors | Apparently, pH, heavy metals content, and the presence of organic chelating substances mediate coliform disappearance rates. Importance of each, however, is poorly understood at present. Salinity strongly enhances the effect of solar radiation. |

A number of researchers have determined values for the half saturation constant ($K_s$) for E. coli growth, using the Monod expression:

TABLE 8-2. COLIFORM BACTERIA FRESHWATER DISAPPEARANCE RATES MEASURED IN SITU (AFTER MITCHELL AND CHAMBERLIN, 1978)

| System | Temperature | k(1/hr) | Reference |
|---|---|---|---|
| Ohio River | Summer (20°C)<br>Winter (5°C | 0.049<br>0.045 | Frost and Streeter (1924) |
| Upper Illinois River | June-September<br>October and May<br>December-March<br>April and November | 0.085<br>0.105<br>0.024<br>0.043 | Hoskins et al. (1927) |
| Lower Illinois River | June-September<br>October and May<br>December-March<br>April and November | 0.085<br>0.037<br>0.026<br>0.029 | Hoskins et al. (1927) |
| "Shallow Turbulent Stream" | | 0.63 | Kittrell and Kochtitzky (1947) |
| Missouri River | Winter | 0.020 | Kittrell and Furfari (1963) |
| Tennessee River (Knoxville) | Summer | 0.043 | Kittrell and Furfari (1963) |
| Tennessee River (Chattanooga) | Summer | 0.005 | Kittrell and Furfari (1963) |
| Sacramento River | Summer | 0.072 | Kittrell and Furfari (1963) |
| Cumberland River | Summer | 0.23 | Kittrell and Furfari (1963) |
| Glatt River | | 1.1 | Wasser et al. (1934) |
| Groundwater Stream | 10°C | 0.021 | Wuhrmann (1972) |
| Leaf River (Mississippi) | | 0.017 | Mahloch (1974) |
| Wastewater Lagoon | 7.9-25.5°C | 0.00833-0.029 | Klock (1971) |
| Maturation Ponds | | 0.083 | Marais (1974) |
| | 19°C | 0.07 | |
| Oxidation Ponds | "T" | $k = 0.108 \cdot (1.19)^{T-20}$ | Marais (1974) |
| Lake Michigan | 10-17°C | 0.36 | Zanoni et al. (1978) |
| Ford Lake (Ypsilanti, Michigan) | August | 0.4 | Gannon et al. (1983) |
| DeGray Reservoir (Arkansas) | October 1976 (15°C) | 0.052 | Thornton et al. (1980) |
| | March 1977 (10°C) | 0.109 and 0.016 | |
| | June 1977 (20°C) | 0.138 and 0.114 | |

Modified from Mitchell and Chamberlin (1978).

$$\mu = \frac{\mu_M S}{K_s + S} \qquad (8\text{-}3)$$

where $\mu$ = growth rate at nutrient concentrations, $day^{-1}$
$S$ = concentration of growth limiting nutrient, mg/l
$\mu_M$ = maximum growth rate, $day^{-1}$
$K_s$ = half-saturation constant producing the half-maximal value of $\mu$, mg/l

Table 8-4 shows some reported values for $K_s$.

However, Gaudy et al. (1971) have shown that the Monod expression (Equation 8-3) is not adequate to describe transient coliform growth behavior. Accordingly, as suggested by Mitchell and Chamberlin (1978), the utility of the $K_s$ value is in evaluating which nutrient may be growth limiting rather than in estimating a growth rate, $\mu$.

TABLE 8-3. VALUES FOR COLIFORM-SPECIFIC DISAPPEARANCE RATES USED IN SEVERAL MODELING STUDIES

| System | k @20°C, 1/hr | Reference |
|---|---|---|
| North Fork Kings River, California | .042 | Chen, et al. (1976) |
| Various Streams | .0004-.146 | Baca and Arnett (1976) |
| Lake Ontario | .02-.083 | U.S. Army Corps of Engineers (1974) |
| Lake Washington | .02 | Chen and Orlob (1975) |
| Various Streams | .042-.125 | Hydroscience (1971) |
| Boise River, Idaho | .02 | Chen and Wells (1975) |
| San Francisco Bay Estuary | .02 | Chen (1970) |
| Long Island Estuaries, New York | .02-.333 | Tetra Tech (1976) |

TABLE 8-4. NUTRIENT $K_s$ VALUES FOR ESCHERICHIA COLI (AFTER MITCHELL AND CHAMBERLIN, 1978)

| Nutrient | Medium | T °C | $K_s$ Micromoles | Remarks | Reference |
|---|---|---|---|---|---|
| Glucose | minimal medium | | 22. | | Monod (1942) |
| | | | 19.4 | | |
| | | | 41.7 | | Moser (1958) |
| | | 30 | 405. | | Schultz and Lipe (1964) |
| | | 30 | 550. | | |
| | seawater | 20 | 44. | | Jannasch (1968) |
| Lactose | seawater | 20 | 50. | | Jannasch (1968) |
| | minimal medium | | 111. | | Monod (1942) |
| Phosphate | minimal medium | | 0.7 | uptake study | Medveczky and Rosenberg (1970) |
| | minimal medium | 30 | 17.35 | | Shehata and Marr (1971) |
| Glucose | | 30 | 0.378 | | Shehata and Marr (1971) |

Work on coliforms in the Ohio River by Frost and Streeter (1924) revealed that the log decay rate for coliforms is nonlinear with time. Accordingly, use of a simple decay expression such as Equation (8-1) with a single value of k is only an approximation to the actual disappearance process. Such an approach must, to some extent as a function of time, overestimate and/or underestimate dC/dt. One approach to solving the problem of a time-variable decay rate is to decompose the death curve into two components, each having its own decay rate (Velz, 1970). This approach is predicated upon typical death rate curves such as those shown in Figure 8-2. These curves have essentially two regions, each with its own characteristic slope, and the coliform concentration as a function of time may be defined as:

$$C_t = C_o e^{-kt} + C_o' e^{-k't} \tag{8-4}$$

where $C_t$ = coliform concentration at time t, MPN or count/100 ml
$C_o, C_o'$ = concentrations of each of the two hypothetical organism types, MPN or count/100 ml
$k, k'$ = decay rates for the two organism types, $day^{-1}$

Table 8-5 shows values for $C_o$, $C_o'$, k, and k' for E. coli as estimated by Phelps (1944).

Lombardo (1972), in an effort to more meaningfully model coliforms, has formulated the dynamics of the coliform population plus streptococci with three separate first-order expressions:

$$C_{T_t} = C_{T_o} e^{-k_t t} \tag{8-5}$$

$$C_{F_t} = C_{F_o} e^{-k_f t} \tag{8-6}$$

$$C_{S_t} = C_{S_o} e^{-k_s t} \tag{8-7}$$

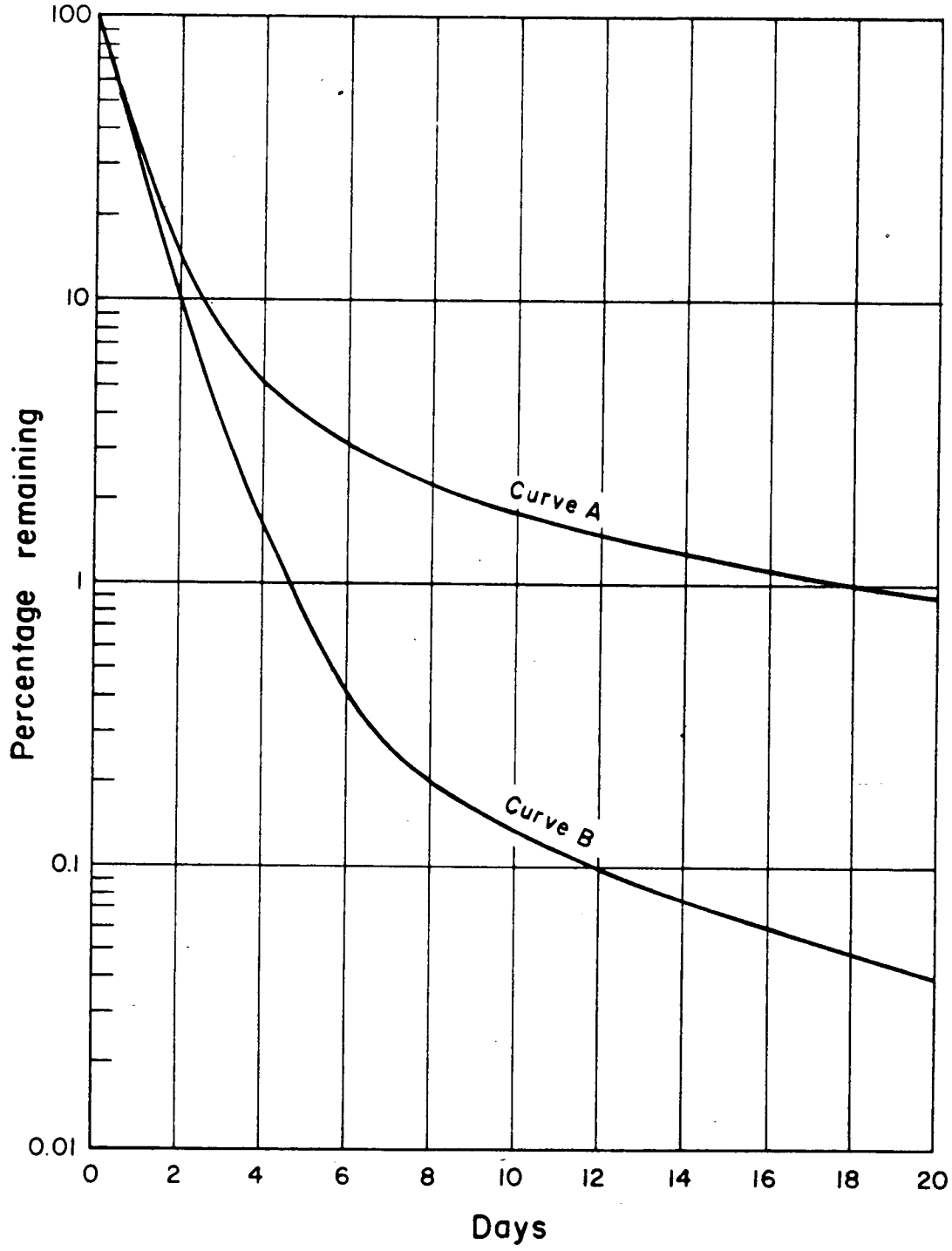


Figure 8-2. Typical mortality curves for coliforms as a function of time. Curve A is for cool weather while curve B represents warm weather decay (redrawn from Velz, 1970).

TABLE 8-5. VALUES OF Co, C', k, AND k' FROM THE OHIO RIVER PHELPS (1944)

| Parameter | Warm Weather | Cold Weather |
|---|---|---|
| $C_o$ (percent) | 99.51 | 97 |
| k (1/day) | 1.075 | 1.165 |
| Half-life (day) | .64 | .59 |
| $C_o'$ (percent) | .49 | 3.0 |
| k' (1/day) | .1338 | .0599 |
| Half-life (day) | 5.16 | 11.5 |

where $C_t$ = organism concentration at time t, MPN or count/100 ml
$C_o$ = organism concentration at time zero, MPN or count/100 ml

Table 8-6 provides data for $k_T$, $k_S$ and $k_F$ as summarized from Lombardo (1972).

As discussed earlier, recent studies have suggested that incident light levels strongly affect coliform disappearance rates. Chamberlin and Mitchell (1978) have defined a light level-dependent disappearance rate coefficient as

$$k' = k_\ell \ell_o e^{-\alpha z} \qquad (8\text{-}8)$$

where k' = the light dependent coliform disappearance rate, 1/hr.
$k_\ell$ = proportionality constant for the specific organism, $cm^2$/cal
$\ell_o$ = incident light energy at the surface, cal/$cm^2$-hr
$\alpha$ = light attenuation coefficient per unit depth
z = depth in units consistent with $\alpha$.

TABLE 8-6. SUMMARY OF DECAY RATES OF TC, FC, AND FS, REPORTED BY LOMBARDO (1972)

| Indicator | n | Median k (1/hr) | Minimum k (1/hr) | Maximum k (1/hr) |
|---|---|---|---|---|
| TC | 16 | 0.038 | 0.010 | 0.105 |
| FC | 13 | 0.048 | 0.008 | 0.130 |
| FS | 5 | 0.007 | 0.002 | 0.063 |

Then, incorporating the vertical dispersion of bacterial cells,

$$\frac{\partial C(z,t)}{\partial t} - V_z \frac{\partial C(z,t)}{\partial z} = E_z \frac{\partial^2 C(z,t)}{\partial z^2} - k'C(z,t) \qquad (8\text{-}9)$$

where $E_z$ = the vertical dispersion coefficient, $cm^2/hr$
$V_z$ = the vertical settling velocity, cm/hr

An expression of this kind is useful where the vertical distribution of coliforms is nonuniform over depth and where disappearance is assumed to be solely a function of light intensity. Chamberlin (1977) has presented solutions of Equation 8-2 for various ranges of $V_z$, $E_z$, $k_\ell$, $\alpha$, and H (depth of water column) using dimensionless variables.

According to an independent development by Mancini (1978) and Chamberlin and Mitchell (1978), if the bacterial cells can be assumed uniform over depth (i.e., the water column is vertically mixed), then the depth-averaged light intensity and the depth-averaged decay rate, respectively, may be computed:

$$\bar{\ell} = \ell_o \left( \frac{1 - e^{-\alpha H}}{\alpha H} \right) \qquad (8\text{-}10)$$

and

$$\bar{k} = k_{\ell}\bar{\ell} \tag{8-11}$$

where $\bar{\ell}$ = the depth-averaged light intensity, cal/cm$^2$/hr

$H$ = the depth of the water column in units consistent with $\alpha$

$\bar{k}$ = the depth-averaged light-dependent disappearance rate, hr$^{-1}$

The depth-averaged, light-dependent, disappearance rate, $\bar{k}$, may be used in the first order disappearance expression for a vertically mixed water body so that:

$$\frac{dC}{dt} = -\bar{k}C \tag{8-12}$$

It is clear that the use of such a model (Equation (8-12)) might be further refined by computing $\bar{k}$ using a sinusoidal function to estimate light levels and incorporating the influence of such factors as latitude, day of the year, time of day, and atmospheric conditions including cloud cover and dust effects. Table 8-7 presents some values for $k_{\ell}$.

Since coliforms and other indicators are known to decay in the dark, Mancini (1978) and Lantrip (1983) have developed decay rate models combining light-dependent and light-independent (i.e., dark) components. The model proposed by Mancini expresses k' as a function of temperature, percent seawater, and depth-averaged light intensity:

$$k' = \frac{(0.8 + 0.006(\%SW))}{24} 1.07^{T-20} + k_{\ell}\bar{\ell} \tag{8-13}$$

where $T$ = water temperature in $^{o}C$.

The model coefficients were estimated based on a combination of laboratory, chamber, and field studies. Note that $k_{\ell}$ is not expressed as a function of either salinity or temperature.

TABLE 8-7. COMPARISON OF $k_\ell$ ESTIMATES BASED ON CHAMBERLIN AND MITCHELL (1978) WITH ADDITIONAL VALUES

| Organism | Study | $k_\ell$ ($cm^2$/cal) | Data Source |
|---|---|---|---|
| Coliform Group | 14 field studies | | Gameson and Gould (1975) |
| | Mean | 0.481 | |
| | 5 percentile | 0.163 | |
| | 95 percentile | 1.25 | |
| | 24 field studies | | Foxworthy and Kneeling (1969) |
| | Mean | 0.168 | |
| | 5 percentile | 0.068 | |
| | 95 percentile | 0.352 | |
| | 61 laboratory studies | | Gameson and Gould (1975) |
| | Mean | 0.136 | |
| | 5 percentile | 0.062 | |
| | 95 percentile | 0.244 | |
| | Estimated from diurnal field experiments in SW | 0.18 at I = 1.0 cal/$cm^2$hr<br>0.07 at I = 0.1 cal/$cm^2$hr | Bellair et al. (1977) |
| Fecal Coliform | Estimated from compilation of field and laboratory studies, both SW and FW. | 0.042 | Mancini (1978) |
| Total Coliforms | 22 chamber studies in FW | | Lantrip (1982) |
| | Mean | 0.004 | |
| | Minimum | 0.000 | |
| | Maximum | 0.013 | |
| Fecal Coliforms | 22 chamber studies in FW | | Lantrip 91982) |
| | Mean | 0.005 | |
| | Minimum | 0.000 | |
| | Maximum | 0.011 | |
| Escherichia coli | 4 field studies | | Gameson and Gould (1975) |
| | Mean | 0.362 | |
| | Minimum | 0.321 | |
| | Maximum | 0.385 | |
| | 4 laboratory studies | | Gameson and Gould (1975) |
| | Mean | 0.354 | |
| Seratia marcescens | 4 field studies | | Gameson and Gould (1975) |
| | Mean | 0.192 | |
| | Minimum | 0.093 | |
| | Maximum | 0.360 | |
| Bacillus subtilis var. niger | 1 laboratory study | 0.002 | Gameson and Gould (1975) |
| Fecal Streptococci | 3 laboratory studies | | Gameson and Gould (1975) |
| | Minimum | 0.048 | |
| | Maximum | 0.123 | |
| | 3 field studies | 0.000 | Gameson and Gould (1975) |
| | 1 field study | 0.007 | |
| | 12 field studies, initial rates | | Foxworthy and Kneeling (1969) |
| | Mean | 0.091 | |
| | Minimum | 0.004 | |
| | Maximum | 0.184 | |
| | 23 chamber studies in FW | | Lantrip (1982) |
| | Mean | 0.008 | |
| | Minimum | 0.001 | |
| | Maximum | 0.028 | |
| Salmonella typhimurium | 2 laboratory studies | 1.48<br>6.40 | Eisenstark (1970) |

Lantrip (1983) developed a set of temperature and light-dependent models based on a series of chamber studies conducted in freshwater. Separate models were determined for TC, FC, and FS. He used nonlinear regression methods to determine the "best" coefficient values and reported both the "best" estimates and associated standard deviations. The three models have the same form:

$$k' = k_{d,20}\theta^{T-20} + k_{\ell}\bar{\ell} \qquad (8\text{-}14)$$

where $k_{d,20}$ = "dark" decay rate at 20°C (1/hr)

$\theta$ = temperature correction term

The coefficients for the three models are summarized in Table 8-8. Note that Lantrip also considers $k_{\ell}$ to be independent of temperature.

Finally, many investigators have noted an initially very low decay rate in laboratory and field studies. For example, see Mitchell and Chamberlin (1978), Mancini (1978), and others. Kapuscinski and Mitchell (1983) and Severin et al. (1978) have argued that this "shoulder" in the decay curve is not the consequence of growth or particle breakup but is instead due to the nature of the photo-oxidation process. Severin et al. present two mechanistic models that would produce a "shoulder":

- Multi-target model based on assumption that several targets or sites in the organism must be hit before the organism will be killed:

$$C_t = C_o\left(1 - \left[(1-e^{-k_{\ell}\cdot\ell\cdot t})^{j}\right]\right) \qquad (8\text{-}15)$$

where j = number of critical sites,

- A series-event model that assumes that the same target must be hit a series of times before inactivation occurs:

$$C_t = C_o e^{-k_\ell \cdot \ell \cdot t} \left( \sum_{i=0}^{n} \frac{(k_\ell \cdot \ell \cdot t)^i}{i!} \right) \qquad (8\text{-}16)$$

where n = event threshold for inactivation

Such models are still novel in engineering applications and have not yet been incorporated into water quality models.

### 8.3.3 Methods of Measurement

Estimates of the coliform disappearance rate, k, may be obtained in a number of ways in the laboratory chamber studies, or, preferably, in situ. For laboratory estimates, samples of effluent may be taken along with samples of receiving water. Then, under controlled conditions of light, temperature, and dilution, the time rate of disappearance may be determined for various combinations of conditions. Unfortunately "bottle effects" often distort laboratory results as shown by Zanoni and Fleissner (1982),

TABLE 8-8. PARAMETER ESTIMATES FOR LANTRIP (1983) MULTI-FACTOR DECAY MODELS

| Indicator | | n | Standard Error Regression | $k_{d,20}$ (1/hr) | $\theta$ | $k_\ell$ ($cm^2$/cal) |
|---|---|---|---|---|---|---|
| TC | Estimate | 38 | 0.0151 | 0.0301 | 1.0893 | 0.0022 |
| | Standard Error | | | 0.0044 | 0.0208 | 0.00065 |
| FC | Estimate | 41 | 0.020 | 0.0305 | 1.0978 | 0.00377 |
| | Standard Error | | | 0.0057 | 0.0280 | 0.00081 |
| FS | Estimate | 38 | 0.0183 | 0.0294 | 1.0859 | 0.00502 |
| | Standard Error | | | 0.0050 | 0.0234 | 0.00076 |

since enteric bacterial growth is promoted by availability of surfaces for attachment.

In situ k values can be determined whether the flow regime is well defined or not, although there are inherent errors involved in each method. Where there are no flow regime data, or where flows are of a transient nature, a commonly used method (e.g., Zanoni et al. 1978 and Gannon et al. 1983 provide recent examples) is to add a slug of a conservative tracer substance (a dye, rare element, or radioisotope) to the steady-state discharge. Then the discharge plume is sampled, dilution is estimated from concentrations of tracer, and the dilution corrected coliform counts permit k to be estimated. It should be recognized that this technique may give misleading results where the dilution of the tracer is due to mixing with water heavily contaminated with the same discharge. Since the tracer had been introduced as a slug, there is no way to know how much of the surviving coliforms originated in the tracer-dosed effluent and how much came from pre-dosing or post-dosing effluent. However, where the flow regime is sufficiently predictable and stable to assure that dilution occurs essentially with ambient water, and where coliform levels in the ambient water are known, this should not be a problem.

Another method, which is particularly useful where discharge is to a channel, is as follows. First, a base sampling site is established below the discharge where the water column is fully mixed normal to the direction of flow. Then samples are taken at the base site and at several points downstream. Based upon known velocities and the change in coliform concentration with distance (time), k values may be estimated. Clearly, errors will be introduced to the extent that there is incomplete lateral mixing of the stream, nonuniform longitudinal velocities laterally and vertically across the channel, and unknown inflows causing dilution or introducing additional coliforms between sampling sites.

Also, sampling can be done so that the same "parcel" or water is sampled, in case the discharge is not at steady-state. For example, if the first sampling site is one mile below the base site, and the channel flow

has a mean velocity of 2 ft per second, then the first sampling site should be sampled:

$$\frac{5280 \text{ ft}}{\text{mile}} \times \frac{1 \text{ second}}{2 \text{ ft}} \times \frac{1 \text{ hr}}{3600 \text{ seconds}} = .73 \text{ hr}$$

or 44 minutes after sampling at the base site. Clearly, however, this does not account for dispersion, and the 44 minutes is an average figure corresponding to the peak loading. Where possible, dye studies or other techniques should be used to characterize stream dispersion at the sampling location. Then, by integrating under the curve, total surviving coliforms can be estimated. If, on the other hand, discharge and stream conditions are clearly at steady-state, sampling times are of no consequence.

Equation (8-17) may be used to estimate k where a slug dose of tracer has been introduced into the discharge (assuming first-order decay):

$$k = -\ln (C_t F_o / F_t C_o)/t \qquad (8\text{-}17)$$

where $F_o$ = discharge concentration of tracer, mg/l
$F_t$ = observed concentration of tracer, mg/l

If no tracer is used and conditions approximating plug flow exist, then:

$$k = -\ln(C_t/C_o)/t \qquad (8\text{-}18)$$

where $C_o$ = concentration of coliforms at the base sampling site, MPN or count/100 ml

Regardless of the technique used for estimating k, it is important to concurrently quantify, to the extent possible, those variables which influence k. For example, light levels should be measured or at least estimated over the period for which k is estimated. If this is not done, and if the effects of the important parameters are not taken into account in modeling coliforms, serious errors will result. Table 8-9 shows how serious such errors can be. The data show T-90 values for coliforms as a function

TABLE 8-9. EXPERIMENTAL HOURLY T-90 VALUES
(AFTER WALLIS, ET AL., 1977)

| Time of Day | T-90 (hours) | Time of Day | T-90 (hours) | Time of Day | T-90 (hours) |
|---|---|---|---|---|---|
| 0100 | 40 | 0900 | 3.2 | 1700 | 5.3 |
| 0200 | 40 | 1000 | 2.5 | 1800 | 6.7 |
| 0300 | 40 | 1100 | 2.3 | 1900 | 8.5 |
| 0400 | 40 | 1200 | 2.5 | 2000 | 11 |
| 0500 | 40 | 1300 | 2.9 | 2100 | 14 |
| 0600 | 19 | 1400 | 3.3 | 2200 | 20 |
| 0700 | 8.0 | 1500 | 3.9 | 2300 | 27 |
| 0800 | 4.6 | 1600 | 4.6 | 2400 | 34 |

of incident light. T-90 values are the times required for 90 percent mortality. The associated k values are .058 $hr^{-1}$ in the dark and .1 $hr^{-1}$ at midday. It is clear that estimating a single value for a k could result in greater than order-of-magnitude errors.

## 8.4 SUMMARY

The coliform group is of interest as an index of potential pathogen contamination in surface waters and has become one of the more commonly modeled water quality parameters. Modeling coliforms usually involves the use of a simple first-order decay expression to describe disappearance. Since regrowth is generally neglected, no growth terms are normally included in the model.

The disappearance rate, k, is a function of a number of variables, the effects of all of which are not well understood. It now appears that light (in the near-UV and visible range) is important as are a number of

physicochemical factors. Rates of disappearance are also sensitive to the salinity of the water which also affects the influence of light on disappearance rates.

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